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Metabolomics: building on a century of biochemistry to guide human health

J. Bruce German[a,b], **Bruce D. Hammock**[c], and **Steven M. Watkins**[d,*]
aDepartment of Food Science and Technology, University of California, Davis, CA, 95616, USA

bNestle Research Center, Lausanne, Switzerland

cDepartment of Entomology and University of California Davis Cancer Center, Davis, CA, 95616, USA

dLipomics Technologies, Inc., 3410 Industrial Boulevard, Suite 103, West Sacramento, CA, 95691, USA

Abstract

Medical diagnosis and treatment efficacy will improve significantly when a more personalized system for health assessment is implemented. This system will require diagnostics that provide sufficiently detailed information about the metabolic status of individuals such that assay results will be able to guide food, drug and lifestyle choices to maintain or improve distinct aspects of health without compromising others. Achieving this goal will use the new science of metabolomics – comprehensive metabolic profiling of individuals linked to the biological understanding of human integrative metabolism. Candidate technologies to accomplish this goal are largely available, yet they have not been brought into practice for this purpose. Metabolomic technologies must be sufficiently rapid, accurate and affordable to be routinely accessible to both healthy and acutely ill individuals. The use of metabolomic data to predict the health trajectories of individuals will require bioinformatic tools and quantitative reference databases. These databases containing metabolite profiles from the population must be built, stored and indexed according to metabolic and health status. Building and annotating these databases with the knowledge to predict how a specific metabolic pattern from an individual can be adjusted with diet, drugs and lifestyle to improve health represents a logical application of the biochemistry knowledge that the life sciences have produced over the past 100 years.

Keywords

metabolomics; metabolic profiling; human health

1. Introduction

Understanding the biochemical pathways that comprise human metabolism represents one of the major achievements of research in the biological sciences over the past 100 years. The enzymes, cofactors, substrates, products and intermediates throughout the multitude of molecular pathways are more completely understood than almost any other aspect of human biology. Four generations of intensive and groundbreaking research established not only the major endogenous biochemical pathways, but also individual enzyme kinetics and the many factors that influence pathway fluxes. This knowledge base has been a major asset to improving the human condition through the 20th century by serving as a resource for the development of

*To whom correspondence should be addressed. E-mail: swatkins@lipomics.com

foods, drugs and agricultural chemicals targeting improved health. A key example of how biochemistry transformed public health is the discovery of the essential nutrients required by humans and the amelioration of these deficiencies in the developed world via an improved food supply (Carpenter, 2003a, b). This assembled knowledge serves as the basis of modern pharmaceutical, pesticide and biotechnology industries, all of which are providing great value to health and agricultural enterprise.

Today, healthcare is faced with a new set of challenges, and knowledge of biochemistry will serve a renewed purpose. Rather than nutritional deficiencies, the major health problems of wealthy countries are those of deranged metabolism. For individuals to remain in good health, all of their metabolic pathways must function appropriately and metabolic needs must be balanced by nutritional inputs. Increasingly, individuals are findings themselves out of metabolic balance, and metabolic diseases, including atherosclerosis, obesity, diabetes, hypertension and osteoporosis, are epidemic in the population (Alberti, 2001). The healthcare system at all levels is being recruited to meet these challenges, and once again, the knowledge of biochemistry will be critical to the success of this effort (Puska, 2002). Biochemistry, however, must be coupled to new tools if the complex and multi-factorial nature of chronic metabolic diseases is to be understood and solved. Although metabolic diseases are often thought of with reference to their hallmark metabolites (diabetes and glucose, cardiovascular disease and cholesterol), biochemical pathways do not exist as isolated systems. Rather, they are a part of the integrated whole that, as an ensemble, constitute human metabolism. It is the proper functioning of this ensemble that prevents progress toward development of fullfledged metabolic disease. Our knowledge of biochemistry is both broad and deep; however, this knowledge is fragmented, as was the research on which it is based – research that was largely focused on specific features of enzymes or pathways independent of metabolism as a whole. Optimizing metabolism as a whole to prevent the chronic derangement of metabolism is now the dauntingly complex problem facing this generation of scientists. Fortunately, analytical technologies are improving to the point that most known endogenous metabolites can be measured by one of a few existing analytical platforms (Lamers et al., 2003, Weckwerth, 2003) and computational technologies are beginning to integrate, mine and annotate panmetabolic databases (Hood, 2003). These technologies constitute the rapidly emerging field of metabolomics, and are providing science with its first look at integrated metabolism, and consequently, with its first look at how integrated metabolism affects human health.

Integrated metabolite data does not by itself comprise an understanding of metabolism. To make sense of metabolite data, the metabolites must first be understood in the context of their biochemical pathways (Watkins, 2002, 2003). To explain why the levels of particular metabolites are outside a normal range, and more importantly to predict how to alter inputs – e.g., diet, drugs and lifestyle – to accomplish a selected change in metabolism, an understanding of how pathways and their respective reactions function is required. This is precisely the body of knowledge that the field of biochemistry built throughout the 20th century. Metabolomics is thus ideally situated to coalesce the existing knowledge of biochemistry into a single, comprehensive strategy to address the health challenges facing the modern world.

2. Metabolomics: the tools to link individual phenotype to biological knowledge

The human genome initiative has established a vision for future research and the application of this knowledge resource that includes as a grand challenge establishing the relationships of phenotype to genomics (Collins et al., 2003). Metabolism is a key aspect to phenotype, hence, describing the distribution of metabolites is a next logical step in the elaboration of functional genomics. Individual metabolite analyses have been pursued for decades as a part of the search for biomarkers of health and disease, with cholesterol emerging as the best example of such

approaches. One fundamental lesson from "omics" is that in using modern analytical technologies, it is often as easy to monitor many analytes as it is to measure one. Existing analytical platforms, including mass spectrometry, NMR and chromatographic systems, are wellsuited to producing high content data, and once optimized are capable of producing highly quantitative data on many metabolites simultaneously. Technologies to measure endogenous metabolites accurately and quantitatively already exist (Newman et al., 2002; Castrillo et al., 2003) and can be applied to generate comprehensive data on a variety of classes of metabolites. Here, one distinction should be made. The analytical technology for measuring the macronutrients, including lipids, simple sugars, amino acids, etc., are already robust. However, lower abundance metabolites, including the bioactive products that act at very low concentrations in tissues in signaling functions, will require additional technology development. Importantly, the measurement of subsets of metabolites, obtained either via pathway classes, analytically expedient clusters or subdivided tissues, must produce quantitative data so that databases amassed by these experiments today and in the future can be integrated as directly comparable datasets.

3. Personalized metabolic assessment

Chronic and metabolic diseases are caused by subtle and longterm dysregulations of metabolism that differ among individuals and yet still give rise to ostensibly single disease states (diabetes, cardiovascular disease, etc.). The myriad possible bases of complex disease necessitate that assessment technologies become both personalized and focused on prevention rather than diagnosis, two properties not possessed in abundance by current biomarker assays. Single biomarker based diagnostics for metabolism-based diseases fail to identify why the surrogate is altered in the first place – i.e., plasma cholesterol and plasma glucose concentrations. Hence, biomarkers fail to provide accurate, actionable information about how to intervene in the development of metabolic disease processes. How can one prescribe a course of action to modulate dysregulated metabolism that has a pathological trajectory if the basis of the dysregulation in metabolism is not known? Thus, more than single markers of disease must be measured to provide individual metabolic context, and to enable individual specific diagnoses and prescriptions. The high-content analytical strategy for enabling personalized assessment is being stewarded in large part by the genomics and pharmacogenomics communities. Yet, metabolomics is probably better suited for determining individual metabolic context, which in the end is an integration of individual genetic, nutritional, pharmacological and environmental status.

Currently, healthcare management and research use single molecule surrogate endpoints, or biomarkers of a disease process, in a variety of applications. They are employed to identify those afflicted with a disease, and to evaluate the progress of an individual with a disease state or to monitor the effectiveness of an intervention in treating the disease. This approach has proven useful for diseases such as bacterial infections and overt toxicities, wherein a specific molecule or groups of molecules are hallmarks of the disease state and largely distinctive within the matrix being sampled. However, the model of evaluating health status using biomarkers of disease must be rejected in order to move forward in more effective health management of individuals within a population, especially for metabolic and chronic disease management. Even in diseases where the utility of biomarkers for predicting metabolic disease outcome and for monitoring risk modification is well established – e.g., plasma cholesterol in atherosclerosis and plasma glucose in diabetes (Grundy, 2002) – single biomarkers are incapable of describing the biochemical pathways responsible for the disease. An ideal assessment technology would be capable of not only detecting the presence of metabolites that predict pathology, but would be capable of identifying both the molecular mechanism responsible for the dysregulation and a logical strategy for intervening in the process. Metabolomics or even the profiling of a target

set of biologically relevant metabolites will provide sufficient information to deliver clinically relevant information meeting both of these criteria.

The difference between a biomarker approach and a metabolite profiling strategy is seen in the example of cholesterol. Measuring cholesterol as a biomarker provides a quantitative estimate of disease risk of an individual within a population, yet the single measurement does not provide sufficient information to deduce why that individual accumulates cholesterol, nor does it suggest the appropriate intervention to resolve the problem. Serum cholesterol can be high in the blood of an individual due to several mechanisms, but three are illustrative: (i) the individual can absorb cholesterol inordinately well through the intestine, or (ii) the individual can produce too much cholesterol through endogenous biosynthesis, or (iii) the individual can convert cholesterol to bile acids very slowly. The measurement of total blood cholesterol does not distinguish among these three mechanisms, but if the analytical measurement of cholesterol is extended to include sterols and their metabolites, it is possible to obtain all the needed information. Those who absorb excessive cholesterol hyper-absorb both cholesterol and phytosterols (Lutjohann et al., 1995) and the concentrations of phytosterols in plasma reflect the higher absolute absorption of sterols from the intestine. For these individuals, an intervention strategy that targets intestinal absorption of cholesterol is recommended (Mussner et al., 2002). Those individuals whose hepatic or whole body biosynthesis is excessive exhibit increased concentrations of mevalonate in plasma as a direct quantitative reflection of the flux through the sterol biosynthetic pathways (Yoshida, 1993). For these hyperproducing individuals, treatment with inhibitors of cholesterol biosynthesis is most appropriate. Finally, in those individuals in whom the causal mechanisms of high cholesterol are insufficient bile acid conversion, the cause itself is detected by the amounts of 7-α-hydroxy-4-cholesten-3-one in plasma (Shoda et al., 1997). Although the determination of the mechanistic basis of cholesterol accumulation is not routinely carried out in the clinic, pharmacological interventions capable of treating two bases for hypercholesterolemia exist. The statins as HMGCoA reductase inhibitors target endogenous synthesis and ezetimibe, an intestinal cholesterol absorption blocker, targets cholesterol uptake (Bruckert, 2003). Prescribing both drugs is obviously unnecessary and costly in cases where only one defect exists, and metabolomic diagnostics can be used to fine tune therapy for individuals, thereby increasing the efficacy and safety of the therapy. By accurately diagnosing the metabolic defect underlying a phenotype, the most appropriate drug treatment can be chosen, thus personalizing the intervention correctly.

4. Analytical technologies to acquire quantitative metabolic data

The first objective of metabolomics is to measure all or a substantial fraction of all metabolites within a biological sample and to quantify each relative to an absolute index of the sample (per gram, milliliter, cell count, etc.). Gene transcriptional analyses – transcriptomics – and gene product analyses – proteomics – are each wrestling with the task of simultaneously identifying and quantifying their respective classes of biomolecules – mRNAs and proteins – and for each type of analysis, single platforms are capable of at least approaching this task (Ohlmeier et al., 2004). Even though the complete sets of metabolites in animals are fewer in absolute number than either transcripts or proteins, metabolites span a much wider distribution of molecular compositions and physical properties within single biological samples. As a result, at present there is no single technological platform capable of measuring and identifying all metabolites in a single sample simultaneously, and comprehensive metabolomic data must be assembled by bringing together data from different platforms. Two basic approaches to metabolomic analysis are being employed. The first strategy, NMR spectroscopy, is a top down tactic in that all of the molecules are interrogated simultaneously by properties that they all share, the presence of NMR active hydrogen or carbon (Nicholson and Wilson, 2003). The second approach is a bottom up tactic where metabolites are separated into discrete molecular classes

analyzed by appropriate qualitative methodologies and then reassembled as a complete metabolite dataset. This approach typically uses mass spectrometry or chromatographic techniques for separating, identifying and quantifying the metabolites.

Rather than measuring individual metabolites, NMR profiles all of the proton magnetic resonances inducible within a sample. NMR spectroscopy is a theoretically quantitative, nondestructive analytical technique that is being used to obtain high resolution spectra of a variety of biological samples, including biological fluids, cells and intact tissues (Nicholson et al., 1999). At present this approach, as it is applied, means that metabolites are analyzed quantitatively because the spectrum of proton magnetic resonances are a quantitative reflection of all of the protons in a sample, but the approach is not qualitative in that the literally thousands of different, resolvable resonances are not assigned to specific metabolites. NMR based metabolomics will advance dramatically when libraries of NMR spectra are sufficient to allow complete metabolite assignments within high resolution spectra of biological samples. Although there are independent organizations pursuing this goal, no large consortia (likely to be necessary given the size of the task) have yet emerged to compile such libraries. Therefore, at present researchers treat the NMR resonance peaks as independent, unassigned statistical variables, and high capacity computer algorithms process these data into various clustering and multidimensional mathematical regression solutions. In the current applications of metabolomics (this approach is also referred to as metabonomics when applied to biofluids), samples from different individuals are analyzed by NMR and the unassigned NMR peaks are used as input data to clustering algorithms to establish whether physiological or health states define themselves as unique *n*-dimensional clusters (Brindle et al., 2002). Because the NMR spectra of most biological samples produce data comprised largely of unassigned resonances, the technique is being applied first as a screening tool to identify directions for subsequent research. By identifying specific resonances that differ between samples, investigators are led immediately to pursue these as the targets for more specific analyses. The chemical information that is inherent in the magnetic resonances of protons within biological molecules up to and including their physical and chemical locations within samples means that whatever directions are pursued by metabolic scientists over the next decades, NMR will continue to be a leading tool for metabolite analyses.

A second platform following the top-down approach uses the remarkable precision of modern mass spectrometry to analyze hundreds to thousands of small molecular weight metabolites comprehensively within samples according to their molecular weights. Mass spectrometers are continually improving in terms of sensitivity, accuracy and dynamic mass range, and are now astonishingly selective in their mass estimation (Soga et al., 2003). Mass spectrometry as an analytical platform thus has the capabilities to assign accurate mass identities to thousands of molecules in a single experiment. Furthermore, the sensitivity of mass spectrometry detection systems is such that metabolites that are present at concentrations orders of magnitude lower than are detectable by NMR are easily identified by mass spectrometry. However, neither the absolute nor the relative concentration of these metabolites can be defined within the mass spectrometer due to variations in ionization properties of instruments and molecules. Hence, metabolites measured by current mass spectrometry techniques are not accurately quantified, and the major drawback to these instruments is that they thus produce qualitative and not quantitative metabolite data. This problem would be solved by the inclusion of quantitative internal standards of metabolites that are enriched in stable isotopes. Again, while several investigators are building libraries of such compounds independently, no large consortium has yet emerged to coordinate the development of what will be in the long term a highly valuable and perhaps necessary scientific resource. While semi-quantitative assays are acceptable for discovery phase research, public health or clinical decisions must be made with quantitative data. In the quest for high content assays, quantification cannot be sacrificed if a public health strategy is to be built around the results.

In a distinctly different overall tactic, which is more of a bottom up experimental model, samples are subdivided to obtain accurate measurement of the identity and quantity of each metabolite. However, separate, parallel analytical platforms are necessary to obtain comprehensive metabolite data for a sample (Newman et al., 2002; Lamers et al., 2003). This complementary approach to the study of metabolomics is based on processes of separating a sample into various classes of metabolites and measuring each class using an appropriate analytical platform (LC–MS, GC–MS, etc.). The entire metabolome is then reassembled into a dataset that is quantitative and internally consistent. The advantages of this approach are that each metabolite is identified and ideally, accurately quantified. The disadvantages of this approach are that it is currently expensive, time intensive and requires a large investment in multiple analytical platforms through which samples are run in parallel.

5. Data requirements for metabolomic databases

As is true for genomics, transcriptomics and proteomics, the great scientific power of metabolomics arises from its ability to produce complete compositional representations of individual biological samples that can be compared one with another. A major strategic goal in all the life sciences is to understand the mechanistic basis for biological differences – e.g., healthy versus diseased, competitive versus noncompetitive organisms, pathogenic versus nonpathogenic microorganisms, etc. – and the ability to compare all aspects of the complete range of metabolites among samples is immensely powerful. However, because most metabolites are present to some degree in virtually all tissues and fluids, it is only a difference in concentration of metabolites that distinguishes key biologically important differences in phenotype/outcome. Therefore, it is vital that metabolite data be quantitative to be suitable for producing databases that provide unbiased biological information about a sample, and that can be used to compare different samples, phenotypes or outcomes, to fit metabolic models and to assess the integrated flux through all metabolic pathways (Watkins et al., 2002; Fiehn, 2003). Such data can continue to be compared, mined and fitted to pursue various hypotheses, including those that are unrelated to the basic hypothesis that was used to produce the data in the first place. These databases are therefore permanent and are not obsolesced by new analytical platforms as they are developed. It is particularly important that databases used to develop predictive measures of health in specific experimental situations are quantitatively absolute so that high throughput, portable platforms can be put in place to assess metabolic health of individuals in the general population.

6. Assessing both substrate availability and enzyme activity

Metabolomic analysis of accessible biofluids provides information on all tissues that deliver to and obtain metabolites from those fluids. Because blood is a central reservoir integrating across the entire organism, for most applications of routine health, it will remain the reservoir of metabolic assessment. Due to the central role of blood in transporting metabolites, metabolic profiling is capable of simultaneously recognizing metabolic status and suggesting optimal strategies for intervention in metabolic disorders. It is possible to distinguish, for example, whether a disorder is due to substrate imbalances and most appropriately resolved by altering substrate abundance through foods, or if an imbalance is due to catalytic activities and is more appropriately resolved by drugs and therapeutics that act on the regulation of biosynthetic pathways themselves. Several examples of effective treatment of pathology by either drugs or foods exist already. For example, unbalanced production of bioactive eicosanoid compounds from essential fatty acids as substrate can promote abnormally high blood clotting rates in certain populations, and contribute to stroke and heart attack (Marcus et al., 2002). In large part, this hyperactive clotting and pro-inflammatory status is the result of increased thromboxane and PGE2 production. These compounds are oxygenated products of arachidonic acid (20:4*n*-6) produced by cyclooxygenase. Alternatively, when the 20 carbon *n*-3 analogue

of arachidonic acid, eicosapentaenoic acid (20:5*n*3), is used as substrate by cyclooxygenase, the products, including PGE3, are antiinflammatory and anticlotting. The two fatty acids, 20:4*n*6 and 20:5*n*3, are competitive for cyclooxygenase, hence, the balance of *n*6 and *n*3 fatty acids in the diet can profoundly alter the balance of pro- and anti-inflammatory compounds produced. Thus, a nutritional approach to reducing inflammation is capable of changing the ratio of pro- and anti-inflammatory compounds produced by cyclooxygenase. However, nutrition alone does not obviously alter the activity of cyclooxygenase, and therefore does not have an impact on the quantity of cycloogenase products produced. If the target of therapy is to change the absolute quantity of eicosanoids that are produced by cyclooxygenase, then pharmacological strategies must be employed. Thus, there are two mutually independent means of modulating inflammatory compound production. The first, substrate balance or nutrition, can modulate the ratio of pro and anti-inflammatory compounds produced. The second, activity modulation of drug therapy, can modulate the number of active molecules produced. Importantly, a quantitative metabolomics strategy can distinguish between these two conditions, and can therefore inform clinicians, patients and researchers as to logical and appropriate strategies for intervention.

7. Metabolic assessment

Assessment is clearly the missing link between current healthcare practice and an individualized approach to health management. Because we lack high-content, highly accurate assessment tools for individuals, public health strategies cannot act on the link between metabolism and health status. It is only possible to wait until deranged metabolism is finally expressed as diagnosable disease to intervene. Similarly, and due to this same lack of assessment of metabolism, it is not possible to interpret whether interventions – food, drugs, exercise, etc. – are safe and effective by resolving the inherent metabolic cause, only that the symptoms diagnostic for the endpoint disease are relieved. Better assessment tools would improve both individual health and grow commercial markets. Drugs would increase in value because their safety and efficacy could be assured, but so also would effective foods and lifestyles. Cholesterol testing again provides such an example of the breadth of values that individual assessment provides across many different commercial enterprises. Individuals are healthier because they can act on the results of direct measures of their personal cholesterol concentrations. Since individuals and their clinicians can detect the results of any action based on the assessment results, companies sell diagnostic kits, drugs, foods, food advice, lifestyle and lifestyle advice on the basis of the measurement and its implications. As more assessment tools are created, new markets will emerge. Public support has the responsibility of preparing the knowledge on which the assessment technologies are based. The ability to use this knowledge to create health value will drive the research for, and application of, assessment technologies.

8. Verifiable health claims

The US Food and Drug Administration has provided a positive incentive to pharmaceutical companies for many years to move from clinical trials based on dose toward trials based on blood concentration. One could provide a further incentive by basing clinical trials on achieving the desired change in metabolomic measurements. Such procedures would reduce the number of patients needed in a trial, reduce the likelihood of failure of the trial, reduce ultimate cost of drug development, and of course, result in better patient care. The cost of the additional analytical data will be offset by these huge benefits, and the analytical technology could be developed to generate multiple new markets based on patient assessment. Just as the establishment of accurate diagnostics opens new therapeutic markets (cholesterol lowering drugs are a $21 billion a year market, enabled by the cholesterol test and the biological

knowledge of cholesterol metabolism), food claims and their regulatory scrutiny would be enabled by accurate and verifiable links between nutrition and metabolic health.

10. Summary

This paper addresses the role of metabolic profiling (metabolomics) in the future of nutrition and health. The potential for metabolomics to bring the assembled knowledge of biochemistry to bear in the quest to achieve fully personalized and preventive health care is profound. The bases for most diseases are found in faulty enzyme activity (genetics, toxicology), improper substrate balance (nutrition) or faulty metabolic regulation (genetics, nutrition, lifestyle, etc.), yet all of these influences are acting to derange normal metabolism. All of these effects are observable through quantitative metabolic assessment, i.e., metabolomics. Not only are changes in metabolism observable, the means to interpret these changes according to specific metabolic pathways is largely at hand precisely because the knowledge of biochemical pathways has been a focused research goal of the life sciences for over a century. The value of this knowledge resource is that observable, metabolic phenomena are potentially understandable and ultimately treatable from an informed perspective. If substrate imbalances are primarily responsible for deranged metabolism, then nutritional intervention is a logical strategy for therapy. Likewise, if enzyme activities are responsible, then drugs that target the appropriate activity can be employed. By measuring metabolites comprehensively, treatments can be tailored to the molecular basis for the disease processes and not simply the endpoint symptoms of the disease's consequences. This informed perspective will eventually provide clinicians and individuals alike with actionable information for managing their health.

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